

The University of Chicago

STUDIES ON THE GROWTH-PRO-
MOTING AND GONAD-STIMULATING
FACTORS OF THE ANTERIOR LOBE
OF THE PITUITARY GLAND

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF MEDICINE

1933

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ERNEST QUONG KING

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THE PHYSIOLOGICAL EFFECT OF THE ADMINISTRATION OF
HEBIN TO THE FEMALE MACACUS RHESUS

The object of this investigation was the study of the effect of the administration of the gonad stimulating factor of the anterior pituitary gland to the macacus rhesus monkey. This specie of animal was chosen chiefly because it has a menstrual cycle which in many ways, including menstrual bleeding at regular monthly intervals simulates that of the human being.

The gonad stimulating factor named "hebin" by Wallen-Lawrence and van Dyke (1931, 1933) was extracted and assayed by their method from the whole pituitary gland of the sheep.

Protocols

Macacus rhesus no. 1. Immature female, weight 1.885 kg. Over a period of 2 years and 5 months this monkey had not been observed to menstruate or grow. By exploratory laparotomy the ovaries were found to be small and immature in appearance. A total of 300 rat units of hebin were given by daily intramuscular injections of 37.5 rat units for eight days. On the 6th day of injection, a vaginal mucous discharge was present. The 8th day, the sexual skin was markedly hyperemic and edematous, similar to the type found at puberty with the onset of sexual activity in this specie of animal. An exploratory laparotomy was performed on the 9th day and the ovaries were found to be moderately enlarged and translucent with ripening follicles. The following day the animal died from peritonitis. At autopsy the ovaries were found to be 9 x 8 x 5 mm., the size of mature ovaries. Gross sec-

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tion showed numerous developing follicles with well developed cumuli. Two ova were removed therefrom, one having a zona pellucida and the second being in a good state of preservation with the nucleus intact. Microscopically numerous large follicles up to 2 mm. in diameter were present, one of which was hemorrhagic and had a very thick membranosa granulosa. This condition was not found in normal ovaries. There appeared to be as many primordial follicles present as one sees normally. The uterus had a very simple interval type of mucous membrane. The stroma resembled that of the repair state but the ground substance did not give a mucoid reaction. There appeared to be a hyperplasia of the blood vessels, capillaries and arterioles.

Macacus rhesus no. 2. Immature female, weight 1.510 kg. Over a period of 2 years and 3 months this monkey had not been observed to menstruate or grow. Exploratory laparotomy revealed that the ovaries were small and immature in appearance. Two days later, daily intramuscular injections of 37.5 rat units of hebin were started and continued for eight days, a total of 300 rat units being given. On the 6th day, mucous vaginal secretion was present and on the 8th day, the sexual skin was hyperemic and edematous. On the 9th day, the first after cessation of hebin injections, exploratory laparotomy was performed and the ovaries were found to be similar to those of macacus rhesus no. 1. A menstrual-like bleeding was found on the 11th day after cessation of hebin treatment. By laparotomy the ovaries were found to be smaller but not markedly changed from the condition found at the second examination. There was no evidence of corpora lutea. The uterus was enlarged and of a firm consistency. A section therefrom showed a superficial hemorrhagic mass of necrotic tissue with a denuded surface. The glands were simple in form with

apparent hyperplasia of the arterioles. Uterine bleeding continued for 4 days. 80 days after hebin injections, the animal was accidentally killed and the ovaries were found to be grossly, small, white and infantile in appearance. The right ovary measured $9 \times 3.8 \times 3.1$ mm.; the left ovary $7.7 \times 4 \times 2$ mm. There were at least six follicles at least 1.0 mm. in diameter in the medulla of the ovary and considerable development of the cortical layer. The mucous membrane of the uterus had glands, simple and secreting in type.

Macacus rhesus no. 3. Immature female, weight 3.125 kg. No history available. By exploratory laparotomy the ovaries were found to be normal, quiescent and pale in appearance. The right ovary was removed. Four days later, daily intramuscular injections of 25 rat units of hebin were started and continued for nine days, except for the 8th when the injection was made subcutaneously. A total of 225 rat units of hebin was given. By the 6th day, hyperemia and edema of the sexual skin were present. On the first day following cessation of treatment, the remaining left ovary was found to be enlarged to about three times its original size and to contain numerous follicular cysts with no evidence of corpora lutea. The ovary was left in situ. The hyperemia and edema of the sexual skin rapidly disappeared following the discontinuance of treatment. Menstrual-like uterine bleeding commenced on the 6th day after cessation of hebin treatment and continued for 13 days. On the 4th day of bleeding, a laparotomy was performed and the ovary was found to be markedly decreased in size. There was no recurrence of sexual activity.

Macacus rhesus no. 4. Mature female, weight 3.175 kg. She had been known to menstruate once. Under observation for two years, she had lost weight and was apparently ill. An exploratory

laparotomy was performed and the ovaries were found to be the size of mature ovaries. The right ovary was removed and weighed 200 mgm. The uterus was soft and pale. Four days later, daily intramuscular injections of 25 rat units of hebin were started and continued for seven days. The 8th day, 25 rat units were given intravenously, on the 9th day 12.5 rat units were given intravenously and 12.5 rat units subcutaneously for a total of 225 rat units. The left ovary as noted by rectal-abdominal examination, gradually increased in size. Only slight hyperemia and no edema of the sexual skin were found with treatment. At laparotomy on the first day following cessation of treatment, the remaining left ovary was found to be about three times its original size and follicular in appearance. Following cessation of treatment, the ovary rapidly decreased in size and there was no evidence of uterine bleeding found at any time.

Macacus rhesus no. 5. Immature female, weight 2.110 kg. All deciduous teeth were present. No operation was performed on this animal. Rectal-abdominal examination revealed that the ovaries and uterus were small and immature in size. 20 rat units of hebin were given intramuscularly for 13 consecutive days for a total amount of 260 rat units. By the 4th day of treatment, the vaginal lavage contained mucus and squamous epithelial cells, which increased in amount as treatment progressed. By the 6th day the ovaries were slightly enlarged, and by the time treatment was discontinued, they were the size of mature ovaries. The enlargement was about equal for both ovaries. Edema and hyperemia of the sexual skin became prominent on the 7th day and increased with treatment. Gross bleeding, moderate in amount occurred the 5th day after cessation of hebin treatment, and continued for ten days. On the cessation of treatment the edema and hyperemia of the

sexual skin rapidly subsided and the ovaries became smaller but did not return to their original size. 36 days after the commencement of the first bleeding there was a moderate reddening and development of edema of the sexual skin which was also accompanied by vaginal findings of mucus and epithelial cells and leucocytes. This condition lasted five days and then subsided with no signs of either gross or microscopic bleeding. On the 47th day, a second series of hebin treatment was started and 100 rat units were given by daily intramuscular injections of 10 rat units. There was a moderate development of hyperemia and edema of the sexual skin but not as marked as that found during the first treatment. Uterine bleeding, moderate in amount occurred two days after cessation of treatment and continued for five days. During the ensuing two weeks the findings were negative.

Macacus rhesus no. 6. Immature female, weight 2.140 kg. Deciduous teeth were all present. The preliminary findings and treatment were identical to those of macacus rhesus no. 5. Mucus was found in the vaginal lavages on the 4th day of treatment and epithelial cells were present on the 6th day. Edema of the sexual skin commenced on the 7th. Gross uterine bleeding was present on the 3d day following cessation of hebin treatment and continued for 19 days, being moderate in amount at the onset and gradually decreasing. Aside from the uterine bleeding described there was no evidence of sexual activity, until the 49th day after the commencement of bleeding, a second series of hebin treatment, similar to those given macacus rhesus no. 5 were given, a total of 100 rat units of hebin being administered intramuscularly in ten days. There was only a moderate development of hyperemia and edema. Uterine bleeding occurred on the second day after cessation of treatment and continued for six days. There was no evidence of

sexual activity during the ensuing two weeks.

Macacus rhesus no. 7. Immature female, weight 2.280 kg. All deciduous teeth were present. Other findings were negative. Treatment was similar to that of *macacus rhesus* no. 5, 260 rat units of hebin being given by daily intramuscular injections of 20 rat units for 13 days. By the 4th day there was mucus and an increased number of leucocytes in the vaginal lavages. On the 8th day, hyperemia and edema of the sexual skin were present and increased until the cessation of treatment. The ovaries gradually increased in size, the right becoming markedly larger than the left, until the close of treatment after which they became smaller, but remaining as large as the ovaries of a mature monkey. Uterine bleeding occurred the 4th day after cessation of treatment and continued for ten days. On the 47th day after commencement of the first uterine bleeding, the sexual skin again showed activity with the appearance of a moderate degree of edema and hyperemia. Mucus and epithelial cells appeared in the vaginal lavages. Gross bleeding occurred spontaneously six days later and continued for five days, being moderate in amount and less than that of the first bleeding.

Macacus rhesus no. 8. Immature female, weight 2.390 kg. All deciduous teeth were present. The sexual development was immature. 35 rat units of hebin were administered by daily intramuscular injections of 5 rat units for seven days. On the 6th day there was reddening of the sexual skin and edema in the region of the tail. There was very little increase in the size of the ovaries during the period of injection. Mucus and epithelial cells appeared in the vaginal lavages on the 6th day. Following cessation of treatment, the edema and hyperemia of the sexual skin subsided and on the 6th day after treatment was completed,

uterine bleeding occurred and continued for five days. There has been no evidence of sexual activity since that time.

Summarizing, the following results were found following the administration of hebin to seven immature and one mature macacus rhesus monkeys: (1) Definite development of ovarian follicles in monkeys 1, 2, 3 and 4. (2) No evidence of luteinizing action, monkeys 1, 2, 3 and 4. (3) Sexual activity characterized by hyperemia and edema of the "sexual skin" similar to that occurring at adolescence, monkeys 1, 2, 3, 5, 6, 7 and 8. (4) When a single ovariectomy was performed, a marked follicular development of the remaining ovary resulted following hebin treatment, which was interpreted as a compensatory hypertrophy, monkeys 3 and 4. (5) Menstrual-like uterine bleeding occurred after cessation of hebin injections in monkeys 2, 3, 5, 6, 7 and 8, and uterine bleeding was repeated after a second series of treatment with hebin in monkeys 5 and 6. (6) Uterine bleeding reoccurred spontaneously the second time without hebin treatment in monkey 7. (7) The follicular development in monkey 4, who had been ill and amenorrheic was not followed by uterine bleeding. (8) Uterine bleeding occurred without signs of ovulation in monkeys 2, 3, 5, 6 and 8.

The findings of Edgar Allen (1927, 1928), Maddux (1930), Morrell (1930) have shown that uterine bleeding can be induced in the macacus rhesus following the cessation of treatment with oestrin. Hartman, Firor and Geiling (1930) have reported stimulation of bleeding in the macacus rhesus following the injection of anterior hypophyseal extracts. Saiki (1932) has also been able to induce uterine bleeding by the use of hypophyseal extracts.

From our investigation it was specifically determined that the hebin prepared by the method of Wallen-Lawrence and

van Dyke was potent in follicle stimulation of the ovaries of the higher primate. It was also capable of inducing uterine bleeding and marked sexual changes in immature animals which was not normally found until the development of puberty in other animals.

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